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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 976 825 A1

# (12) EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:
**02.02.2000 Bulletin 2000/05**

(21) Application number: **98914082.7**

(22) Date of filing: **17.04.1998**

(51) Int. Cl.[7]: **C12N 15/12**, C12N 15/63, C12P 21/02, C07K 14/52, C07K 14/82, C07K 16/24, C07K 16/32, C12Q 1/02

(86) International application number:
**PCT/JP98/01782**

(87) International publication number:
**WO 98/48015 (29.10.1998 Gazette 1998/43)**

(84) Designated Contracting States:
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE**

(30) Priority: **18.04.1997 JP 11640297**

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## (54) TRANSCRIPTIONAL REGULATOR

(57) A gene encoding a novel transcriptional regulator having a bromo domain has been successfully isolated from a human testis cDNA library by effecting polymerase chain reactions employing primers prepared based on an EST sequence which has a high homology with a transcriptional regulatory factor "RING3" having a bromo domain and has been found out by retrieving a data base with the use of the sequence of "RING3". By analyzing the expression of the isolated gene, it has been found out that this gene is expressed strongly in testicular cells with a potent ability to proliferate. The use of the above transcriptional regulator and its gene makes it possible to screen candidate compounds for factors interacting with the transcriptional regulator or drugs controlling the activity of the regulator.

EP 0 976 825 A1

Printed by Xerox (UK) Business Services
2.16.7/3.6

## Description

### Technical Field

**[0001]** The present invention relates to a novel transcriptional regulator containing a bromodomain and a gene encoding it.

### Background Art

**[0002]** The bromodomain is a characteristic motif of amino acids found in transcriptional regulators, and is believed to be involved in interaction with other proteins, such as other transcriptional regulators. Proteins having a bromodomain usually contain one or two (Tamkun, J. W. et al. (1992) Cell,68,561-572, Haynes, S.R. et al. (1992) Nuc. Acids Res., 20:2603), but sometimes as many as five bromodomain motifs (Nicolas, R. H. and Goodwin, G. H. (1996), Gene, 175 (12):233-240). This motif is found in a wide variety of animals. For example, it is identified in yeast (Winston, F. et al. (1987), Genetics, 115:649-656; Laurent, B. C. et al. (1991), Proc. Natl. Acad. Sci. USA, 88:2687-2691), *Drosophila* (Digan, M. E. et al.(1986), Dev. Biol., 114:161-169; Tamkun, J. W. et al. (1992), Cell, 68:561-572), and mammals (Denis, G. V. and Green, M. R. (1996), Genes and Devel., 10: 261-271; Yang, X. J. et al. (1996), Nature, 382: 319-324).

**[0003]** All transcriptional regulators containing a bromodomain serve to control signal-dependent transcription in actively proliferating cells (Tamkun, J. W. et al. (1992), Cell, 68: 561-572; Haynes, S.R. et al. (1992), Nuc. Acids Res., 20:2603). Due to this feature, it is suggested that cancer may develop if the gene for the protein containing a bromodomain is not normally controlled. In fact, several studies have shown that human transcriptional regulators with a bromodomain RING3, p300/CBP, and PCAF may be involved in oncogenesis.

**[0004]** RING3 was identified during an extensive analysis of the sequences of human class II major histocompatibility systems (Beck et al., (1992) DNA Seq. 2: 203-210). The protein encoded by RING3 is homologous to D26362, a human gene (Nomura et al., (1994) DNA Res. 1: 223-229) and fsh, a drosophila gene (Digan et al., (1986), Dev. Biol.,114: 161-169). All three genes encode proteins contain two copies of a bromodomain and a PEST sequences. The bromodomain is a motif consisting of 59 to 63 amino acid residues and is considered to be involved in protein-protein interactions. It is found among the transcriptional regulator proteins (Tamkun, J. W. et al., (1992) Cell,68,561-572; Haynes, S. R. et al., (1992) Nuc. Acids Res. 20: 2603). The PEST sequence is a cluster of proline (P), glutamic acid (E), serine (S) and threonine (T), which characterizes the proteins that undergo rapid proteolysis in the cell.

**[0005]** The protein encoded by RING3 has a molecular weight of 90 kD and has serine-threonine activity (Denis and Green, (1996) Genes and Devel. 10: 261-271). Comparison of the sequences of RING3 and fsh with those of kinase domains of known serine-threonine kinases revealed that the sub-domains of the kinase motif are conserved, though in no particular order (most of them are similar to the corresponding sub-domains of a proto-oncogene c-mos). Kinase activity of RING3 is stimulated by interleukin-1 (IL-1) and forskolin, but not by a certain category of cytokines (Denis and Green, (1996) Genes and Devel. 10: 261-271). A close relationship between kinase activity and growth phase in chronic and acute lymphocytic leukemia suggests the role RING3 plays in the leukemogenesis regulatory pathway (Denis and Green, (1996) Genes and Devel. 10: 261-271). The analysis of the drosophila fsh gene suggested the interaction with the trithorax transcriptional regulator, a possible target for the putative phosphorylative activity of fsh (Digan et al., (1986) Dev. Biol. 114: 161-169; Mozer and Dawid, (1989) Proc. Natl. Acad. Sci. USA 86: 3738-3742). The triathorax gene and its homologue ALL-1 have a C4HC3 zinc finger, a motif commonly found among the genes present at leukemia breakpoints(Aasland et al., (1995) Trends Biochem. Sci. 20: 56-59; Saha et al., (1995) Proc. Natl. Acad. Sci. USA 92: 9737-9741).

**[0006]** In addition to RING3, at least two other bromodomain proteins, p300/CBP and PCAF, are associated with oncogenesis. Although p300 protein and CBP protein are encoded by different genes, they are extremely closely related, and therefore, they are often called p300/CBP. Mutations in CBP are often found in familial and sporadic cancers. Mutations in CBP sometimes result in Rubinstein-Taybi syndrome, which causes patients to develop various malignant tumors (Petrij et al., (1995) Nature 376: 348-51). Furthermore, CBP is fused with MOZ at t(8;6)(p11;p13) translocation (Borrow et al., (1996) Nature Genet. 14:33-41). This fusion protein possibly causes leukemogenesis by its aberrant acetyltransferase activity (Brownwell and Allis, (1996) Curr. Opin. Genet. Devel. 6:176-184). Mutation in p300 is found in sporadic colon and gastric cancers (Muraoka et al., (1996) Oncogene 12: 1565-1569), and p300 has been suggested to be a gene for a tumor- suppressing factor located on chromosome 22q. Another fact that suggests the role of p300/CBP in cancer is that it interacts with the known oncogenes. For example, it is a coactivator of c-Myb (Dai, et al., (1996) Genes and Devel. 10: 528-540) and c-Fos (Bannister and Kouzarides, (1996) Nature 384: 641-643) transcriptional factors, to which the E1A protein of Adenovirus bind (Yang et al., (1996) Nature 382: 319-324). The interaction between E1A and p300/CBP is inhibited by PCAF, a bromodomain protein.

**[0007]** Like p300/CBP, PCAF also has histone acetyltransferase activity. PCAF, when exogenously expressed, can reduce the proliferation associated with E1A in cultured cells (Yang et al., (1996) Nature 382: 319-324). Therefore, one of the first mechanisms of the

activity of the E1A oncogene may be to inhibit the interaction between PCAF and p300.

**[0008]** Thus, it is thought that aberrant regulation of transcriptional regulators containing a bromodomain may be closely related to various diseases, for example, cancer. Transcriptional regulators containing a bromodomain have thus recently received much attention as novel targets for treating cancer.

## Disclosure of the Invention

**[0009]** An objective of the present invention is to provide a novel transcriptional regulator having a bromodomain and DNA encoding said transcriptional regulator. Another objective of the present invention is to provide a vector carrying said DNA, a transformant retaining said DNA, and a process for producing a recombinant protein by utilizing said transformant. A further objective of the present invention is to provide a method of screening a compound that binds to said transcriptional regulator.

**[0010]** To solve the problems described above, the inventors searched a database based on the sequence of RING3 transcriptional regulator with a bromodomain and found several EST sequences highly homologous to RING3. Primers were then prepared based on one of the EST sequences, and polymerase chain reaction was performed in a human testis cDNA library using the primers. As a result, a gene encoding a novel transcriptional regulator with a bromodomain was successfully isolated. By analyzing the expression of this gene, the inventors discovered that the gene is highly expressed in the testis cells. Moreover, the inventors found that a factor interacting with the transcriptional regulator, or a candidate pharmaceutical compound that regulates activity of the transcriptional regulator, can be screened by utilizing the transcriptional regulator and the gene encoding it.

**[0011]** Thus, the present invention relates to novel transcriptional regulators each having a bromodomain and the genes encoding them, and to a method of screening for a related factor or a candidate compound as a medicament using said proteins or genes, and more specifically relates to:

(1) a transcriptional regulator having a bromodomain, which comprises the amino acid sequence shown in SEQ ID NO:1, or said sequence wherein one or more amino acids are substituted, deleted, or added;
(2) a transcriptional regulator having a bromodomain, which is encoded by DNA hybridizing with DNA comprising the nucleotide sequence shown in SEQ ID NO: 2;
(3) DNA coding for the transcriptional regulator according to (1) or (2);
(4) a vector comprising the DNA according to (3);
(5) a transformant expressibly retaining the DNA according to (3);
(6) a method for producing the transcriptional regulator according to (1) or (2), which comprises culturing the transformant according to (5);
(7) an antibody binding to the transcriptional regulator according to (1) or (2);
(8) a method of screening a compound having binding activity to the transcriptional regulator according to (1) or (2), wherein the method comprises

(a) contacting a sample with the transcriptional regulator according to (1) or (2) and
(b) selecting a compound having binding activity to the transcriptional regulator according to (1) or (2);

(9) a compound having binding activity to the transcriptional regulator according to (1) or (2), which can be isolated according to the method of (8);
(10) the compound according to (9), which is naturally occurring; and
(11) DNA specifically hybridizing with DNA comprising the nucleotide sequence shown in SEQ ID NO: 2 and having at least 15 nucleotides.

**[0012]** Here, the term "transcriptional regulator(s)" means protein(s) that control gene expression, and "bromodomain" means an amino acid motif conserved among the transcriptional regulators associated with signal-dependent transcription, wherein said motif is involved in protein-protein interaction.

**[0013]** The present invention relates to a novel transcriptional regulator having a bromodomain. The amino acid sequence of the protein designated TSB contained in the transcriptional regulator of the present invention is shown in SEQ ID NO: 1, and the nucleotide sequence of the cDNA encoding the protein is shown in SEQ ID NO: 2. TSB protein is generally known as a region involved in interaction with other factors including transcriptional regulators, and it has a bromodomain(s) (amino acid positions 49-109 and 292-352 of SEQ ID NO: 1), a characteristic motif of transcriptional regulators involved in cancer (Fig. 1). It is also highly expressed in the testis cells (Example 4). These facts suggest that TSB protein, like other transcriptional regulators having bromodomains, may be involved in cell proliferative diseases and cancers, particularly in testis cancer. In this connection, bromodomains are thought to play an important role. Thus, TSB protein, or a transcriptional regulator functionally equivalent thereto, can be used to prevent and treat cell proliferative diseases and cancers.

**[0014]** The transcriptional regulators of the present invention can be prepared as recombinant proteins generated using a recombinant gene technique, or as naturally-occurring proteins. The transcriptional regulators of the present invention include both recombinant and naturally-occurring proteins. The recombinant proteins can be prepared using a method such as incorporating

DNA encoding a transcriptional regulator of the present invention as described below (e.g. DNA having the nucleotide sequence shown in SEQ ID NO: 2) into a suitable expression vector, which is then introduced into host cells, and purifying the protein obtained from the transformant. The naturally occurring proteins can be prepared using a method such as preparing a column which utilizes an antibody obtained from a small animal immunized with the recombinant protein prepared as above or its partial peptide, and subjecting the extract from a tissue or cells in which a transcriptional regulator of the present invention is overexpressed (e.g. testis) to affinity chromatography using said column.

**[0015]** The present invention also relates to transcriptional regulators functionally equivalent to the transcriptional regulators of the present invention. A method of introducing mutation into amino acids of a protein to isolate such a protein is well known to one skilled in the art. Thus, it is well within the art of an ordinarily skilled person to isolate a protein functionally equivalent to the TSB protein having the amino acid sequence shown in SEQ ID NO: 1 by appropriately modifying, for example, substituting amino acids without affecting the function of the protein using a site-directed mutagenesis system using PCR (GIBCO-BRL, Gaithersburg, Maryland), a site-directed mutagenesis using oligonucleotides (Kramer, W. and Fritz, H. J. (1987), Methods in Enzymol., 154: 350-367), or the similar methods. Mutation in an amino acid of a protein can also occur spontaneously. The transcriptional regulators of the present invention include those having the amino acid sequence (SEQ ID NO: 1) of the TSB protein in which one or more amino acids are substituted, deleted, or added, and functionally equivalent to the TSB protein. The number of mutagenized amino acids is not particularly limited as long as it retains function equivalent to the TSB proiten. It is usually 50 amino acids or less, preferably 30 amino acids or less, more preferably 10 amino acids or less, and most preferably five amino acids or less.

**[0016]** As another method of isolating a functionally equivalent protein utilizing a hybridization technique (Sambrook, J. et al., Molecular Cloning 2nd ed. 9.47-9.58, Cold Spring Harbor Lab. press, 1989) is well known to one skilled in the art. Based on the DNA sequence encoding the TBS protein (SEQ ID NO: 2), or the fragment thereof, a person with ordinary skill in the art can isolate DNA highly homologous to said DNA sequences using a hybridization technique (Sambrook, J. et al., Molecular Cloning 2nd ed. 9.47-9.58, Cold Spring Harbor Lab. press, 1989) to obtain a protein functionally equivalent to the TBS protein. The transcriptional regulators of the present invention include those encoded by DNA that hybridizes with DNA encoding the TBS protein and functionally equivalent to the TBS protein. Source organisms used to isolate a functionally equivalent protein includes, for example, mouse, rat, cattle, monkey, and pig as well as human. The stringency of hybridization for isolating DNA encoding a functionally equivalent protein is suitably determined by one skilled in th art and is typically 42°C, 2×SSC, 0.1% SDS, preferably 50°C, 2×SSC, 0.1% SDS, and more preferably 65°C, 2×SSC, 0.1% SDS. The higher the temperature, th more highly homologous DNA will be obtained. High amino acid homology means usually 40% or more, preferably 60% or more, more preferably 80% or more, or most preferably 95% or more. The transcriptional regulator obtained by the hybridization technique preferably contains bromodomain(s). It can also contain a serine/threonine kinase domain which functions to phosphorylate other proteins, PEST sequence which is a characteristic sequence of the proteins undergoing rapid intracellular proteolysis, and a nuclear transport signal which functions to tranport the protein into nucleus. The presence of the bromodomain in the protein can be identified by searching the bromodomain motif PROSITE database on DNASIS (HITACHI Software engineering).

**[0017]** The present invention also relates to DNA encoding a protein of the present invention. The DNA of the present invention includes CDNA, genomic DNA, and chemically synthesized DNA, but is not limited thereto as long as it codes for a protein of the present invention. cDNA can be prepared, for example, by designing a primer based on the nucleic acid sequence shown in SEQ ID NO: 2 and performing plaque PCR (see Affara, N.A. et al. (1994), Genomics, 22: 205-210). The genomic DNA can be prepared according to a standard technique using, for example, Qiagen genomic DNA kits (Qiagen, Hilden, Germany). The DNA sequence thus obtained can be determined according to a standard technique using a commercially available dye terminator sequencing kit (Applied Biosystems) and the like. In addition to applying to the production of recombinant proteins as described below, the DNA of the present invention may be applied to gene therapy and the like.

**[0018]** The present invention also relates to a vector into which the DNA of the present invention is inserted. The vector of the present invention includes various types of vectors, e.g. for expressing the protein of the present invention *in vivo* and for preparing recombinant proteins and appropriately selected depending on the purpose. Vectors used for expressing the protein of the present invention *in vivo* (in particular, for gene therapy) include the adenovirus vector pAdexLcw and the retrovirus vector pZIPneo. An expression vector is particularly useful for producing a protein of the present invention. Although there is no particular limitation to th expression vectors, the following vectors are preferred: pREP4 (Qiagen, Hilden, Germany) when *E. coli* is used; SP-Q01 (Stratagene, La Jolla, CA) when yeast is used; and BAC-to-BAC baculovirus expression system (GIBCO-BRL, Gaithersburg, Maryland) when insect cells are used. A LacSwitch II expression system (Stratagene; La Jolla, CA) is advantageous when mammalian cells, such as CHO, COS, and NIH3T3 cells, are used.

The DNA of the present invention can be inserted into vectors using a standard method.

**[0019]** The present invention also relates to a transformant expressibly retaining the DNA of the present invention. The transformants of the present invention include one harboring the above-described vector into which the DNA of the present invention is inserted and one having the DNA of the present invention integrated into its genome. The DNA of the present invention can be retained in the transformant in any form as long as the transformant expressibly retains the DNA of the present invention. There is no limitation to host cells into which a vector of the present invention is introduced. If the cells are used to express a protein of the present invention for the purpose of *ex vivo* gene therapy, various cells can be used as target cells suited to diseases. Cells such as *E. coli*, yeast cells, animal cells, and insect cells can be used for producing the protein of the present invention. The vector can be introduced into the cells by methods such as electroporation andthe calcium phosphate method. Recombinant proteins can be isolated and purified from the transformants generated for producing the said proteins according to a standard method.

**[0020]** The present invention also relates to antibodies that bind to the transcriptional regulators of the present invention. The antibodies of the present invention include, but are not limited to, polyclonal and monoclonal antibodies. Also included are antisera obtained by immunizing an animal such as a rabbit with a protein of the present invention, any class of polyclonal or monoclonal antibodies, humanized antibodies generated by gene recombination, and human antibodies. The antibodies of the present invention can be prepared according to the following method. For polyclonal antibodies, antisera can be obtained by immunizing a small animal, such as a rabbit, with a transcriptional regulator of the present invention or a partial peptide thereof, then recovering the fractions that only recognize the transcriptional regulator of the present invention through an affinity column coupled with the transcriptional regulator of the present invention. Immunoglobulin G or M can be prepared by purifying the fractions through a Protein A or G column. For monoclonal antibodies, a small animal, such as a mouse, is immunized with a transcriptional regulator of the invention, the spleen is removed from the mouse and homogenized into cells, the cells are fused with myeloma cells from a mouse using a reagent such as polyethylene glycol, and clones that produce antibodies against the transcriptional regulator of the invention are selected from the resulting fused cells (hybridoma). The hybridoma obtained is then transplanted into the abdominal cavity of a mouse, and the ascites are recovered from the mouse. The obtained monoclonal antibodies can then be prepared by purifying, for example, by ammonium sulfate precipitation through a Protein A or G column, by DEAE ion exchanging chromatography, or through an affinity column coupled with the protein of the invention. Besides being used to purify or detect the transcriptional regulators of the present invention, the antiobodies of the present invention can beused as a drug for suppressing the functions of the transcriptional regulator of the present invention. When an antibody is used as a drug, a human or humanized antibody is effective with regard to immunogenicity. A human or humanized antibody can be prepared according to methods well known in the art. For example, a human antibody can be prepared by immunizing a mouse whose immune system is replaced by a human system with the transcriptional regulator of the present invention. A humanized antibody can be prepared by the CDR grafting method in which an antibody gene is cloned from monoclonal antibody-producing cells and its antigenic determinant site is transplanted to an existing human antibody.

**[0021]** The present invention also relates to a method for screening a compound that binds to transcriptional regulators of the present invention. The screening method of the present invention includes steps of (a) contacting a transcriptional regulator of the present invention with a test sample and (b) selecting a compound that has binding activity for the transcriptional regulator of the present invention. Test samples used for the screening include, but are not limited to, a library of synthetic low molecular weight compounds, a purified protein, an expression product of a gene library, a library of synthetic peptides a cell extract, and a supernatant of the cell culture. Various methods well known to one skilled in the art can be used for selecting a compound binding to a transcriptional regulator of the present invention.

**[0022]** A protein that binds to a transcriptional regulator of the present invention can be screened by West-western blotting comprising steps of generating a cDNA library from the tissues of cells expected to express the protein that binds to a transcriptional regulator of the present invention (e.g. testis) using a phage vector (λgt11, ZAPII, etc.), allowing the cDNA library to express on the LB-agarose plate, fixing the expressed proteins on a filter, reacting them with the transcriptional regulator of the present invention purified as a biotin-labeled protein or a fusion protein with GST protein, and detecting plagues expressing the protein bound to the regulator on the filter with streptavidin or anti-GST antibody (Skolnik, E. Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fisher, R., Drepps, A., Ullrich, A. and Schlessinger, J. (1991), Cloning of PI3 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. Cell 65, 83-90). Alternatively, the method comprises expressing in yeast cells a transcriptional regulator of the present invention which is fused with SFR or GAL4 binding region, constructing a cDNA library in which proteins are expressed in a fusion protein with the transcription activation site of VP16 or GAL4 from the cells expected to express a protein that binds to the transcriptional reg-

ulator of the present invention, introducing the cDNA library into the above-described yeast cells, isolating the cDNA derived from the library from the detected positive clones, and introducing and expressing it in *E. coli*. (If a protein that binds to the transcriptional regulator of the present invention is expressed, a reporter gene is activated by the binding of the two proteins. The positive clones can then be identified.) This method can be performed using Two-hybrid system (MATCHMAKER Two-Hybrid System, Mammalian MATCHMAKER Two-Hybrid Assay Kit, MATCHMAKER One-Hybrid System (all from Clontech); HybriZAP Two-Hybrid Vector System (Stratagene) or in accordance with Dalton, S. and Treisman R. (1992), Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element, Cell, 68: 597-612) Another method is to apply a culture supernatant or a cell extract from the cells suspected to express a protein which binds to the transcriptional regulator of the present invention onto an affinity column coupled with the transcriptional regulator of the present invention, and purify the protein specifically bound to the column.

**[0023]** Also well known to one skilled in the art are a method of screening molecules that bind to a transcriptional regulator of the present invention by reacting the immobilized transcriptional regulator of present invention with a synthetic compound, natural substance bank, or a random phage peptide display library, and a method of isolating low molecular weight compounds, proteins (or their genes), or peptides which bind to a transcriptional regulator of the present invention by utilizing the high-throughput technique of combinatorial chemistry (Wrighton, N. C., Farrell, F. X., Chang, R., Kashuyap, A. K., Barbone, F. P., Mulcahy, L. S., Johnson, D. L., Barrett, R. W., Jolliffe, L. K., Dower, W. J., Small peptides as potent mimetics of the protein hormone erythropoietin, Science (UNITED STATES) Jul. 26, 1996, 273: 458-464; Verdine, G. L., The combinatorial chemistry of nature, Nature (ENGLAND), Nov. 7, 1996, 384: 11-13; Hogan, J. C. Jr., Directed combinatorial chemistry, Nature (ENGLAND), Nov. 7, 1996, 384: 17-19). The compounds thus isolated by the screening method of the present invention are candidates for drugs for enhancing or surpressing the activity of a transcriptional regulator of the present invention. When the compounds isolated by the screening method of the present invention are used as pharmaceuticals, they can be formulated by a known pharmacological process. For example, they can be administered to a patient with pharmaceutically acceptable carriers and vehicles (e.g. physiological saline, vegetable oil, a dispersant, a surfactant, and a stabilizer). The compounds may be percutaneously, intranasally, transbronchially, intramuscularly, intravenously, or orally administered, depending on their properties.

**[0024]** The present invention also relates to DNA specifically hybridizing with DNA coding the TBS protein and having at least 15 nucleotides. As used herein, "specifically hybridizing" means that no cross-hybridization occurs between DNA encoding other proteins under ordinary conditions for hybridization, and preferably under stringent conditions. Such DNA may be used as a probe for detecting and isolating the DNA encoding the TBS protein, and as a primer for amplifying the DNA encoding the protein of the present invention. Specific examples of the primers include those shown in SEQ ID NOs: 3 and 4.

Brief Description of the Drawings

**[0025]**

Figure 1 shows the nucleic acid sequence of TSB aligned with the amino acid sequence of the open reading frame. The three motifs identified by the search of the PROSITE database are underlined. Two bromodomains (amino acid positions 49-109 and 292-352) and a PEST sequence (amino acid positions 636-672) are identified.

Figure 2 compares the amino acid sequences of the predicted kinase domains of TSB, RING3, and fsh. The sub-domains of kinase are disclosed in Denis and Green, (1996) Genes and Devel. 10: 261-271, and sub-domains I-II are excluded. The numerals correspond to the translation position of TSB. The conserved residues are shaded.

Figure 3 shows the map location of TSB. The position is indicated relative to the positions of the adjacent markers on chromosome 1p determined by radiation hybrid analysis.

Figure 4A shows the results of Northern blot analysis of TSB in the normal tissues (Lane 1, heart; Lane 2, brain; Lane 3, placenta; Lane 4, lung; Lane 5, liver; Lane 6, skeletal muscle; Lane 7, kidney; Lane 8, pancreas; Lane 9, spleen; Lane 10, thymus; Lane 11, prostate; Lane 12, testis; Lane 13, ovary; Lane 14, small intestine; Lane 15, colon (mucous lining); and Lane 16, peripheral leukocytes). Figure 4B shows the results of Northern blot analysis of TSB in carcinoma cell lines (Lane 1, promyelocytic leukemia HL-60; Lane 2, HeLa S3 cells; Lane 3, chronic myelocytic leukemia K-56; Lane 4, lymphoblastic leukemia MOLT-4; Lane 5, Burkitt's lymphoma Raji; Lane 6, large intestinal adenocarcinoma SW480; Lane 7, lung carcinoma S549; and Lane 8, melanoma G361). The positions of molecular weight markers are indicated on the right.

Best Mode for Implementing the Invention

**[0026]** The following examples illustrate the present invention in more detail, but are not to be construed to limit the scope of the present invention.

Example 1 Identification of EST homologous to RING3 and isolation of its full-length sequence

**[0027]** A BLAST search of the EST database identified numerous ESTs homologous to the DNA sequence of the RING3 gene (Beck et al., (1992), DNA Seq. 2:203-210) used as a probe. Among these ESTs, EST H64204, which was derived from a testis- specific cDNA library (Diatchenko et al., (1996) Proc. Natl. Acad. Sci. USA 93: 6025-6030), had 65% sequence homology to RING3 spanning 285 bp.

**[0028]** To clone the full-length sequence of EST H64204, PCR primers U (SEQ ID NO: 2: AATGTCTCTGCCAAGTCGACAA) and L (SEQ ID NO: 4: AGCATCCACAGGACGTTGAAAG) were designed to perform PCR using testis cDNAs as templates. PCR was carried out at 94 °C for 8 min. followed by 35 cycles of 94 °C for 30 sec (heat annealing), 60 °C for 1 min (annealing), and 72 °C for 1 min (extension). AmpliTaq gold (Perkin Elmer) was used as an enzyme for PCR. This resulted in a PCR product of 175 bp. Subsequently, a testis cDNA library was screened (Clontech; HL3024a) using this PCR product as a probe. All the probes were labeled with [$\alpha$-$^{32}$P]dCTP by random priming and were purified through Chromaspin (10 columns; Clontech). Hybridization was performed in ExpressHyb hybridization solution (Clontech) for 1 hour at 65°C. The filter was washed at the final stringency of 1×SSC, 0.1% SDS, 65°C. The sequence of a cDNA clone that was aligned with the EST sequence was used to re-screen the library. This process was repeated until a series of overlapped clones giving a full-length sequence of the coding region of the gene was obtained. As a result, a continuous sequence of 3,104 bp encoding 947 amino acids was found in an open reading frame (ORF) at the nucleotide positions 106-2946. The ORF is followed by a short stretch of a 3' untranslated region of 60 bp, which is terminated with a poly(A) tail that has a polyadenylated signal (ATTAAA) in the 20 bp upstream of it. The inventors designated the isolated clone "TSB (testis specific bromodomain). SEQ ID NO: 2 shows the nucleotide sequence of TSB along with the predicted amino acid sequence thereof. The predicted amino acid sequence is also shown in SEQ ID NO: 1. The nucleotide sequences were determined with the automated sequencer ABI377 (Perkin Elmer) by use of ABI dye terminator chemistry.

Example 2 Homology and motifs

**[0029]** RING3 (66% homology, 59% identity spanning 649 amino acids), D26362 (62% homology, 56% identity spanning 649 amino acids), and fsh (62% homology, 56% identity spanning 565 amino acids) were identified as the amino acid sequences having the highest homology with TBS by searching protein databases.

**[0030]** Two bromodomains (amino acid positions 49-109 and 292-352) were identified by searching the PROSITE database for the motif of amino acid sequence. A PEST sequence (Rodgers et al., (1986) Science 234: 364) was also present in amino acid positions 636-672. Figure 1 shows the locations of these motifs.

**[0031]** Since RING3 is also known to have serine-threonine kinase activity (Denis and Green, (1996) Genes and Devel. 10: 261-271), the amino acid sequence of TSB was compared with that of the predicted kinase domain of RING3 using Bestfit at GCG. The result showed that the predicted kinase domain of RING3 is very well conserved in TSB (Fig. 2). However, it was found that TBS did not contain sub-domain I, which codes for the predicted ATP-binding domain, and sub-domain II, which codes for catalytic lysine, suggesting that the kinase activity of TSB was possibly lost.

**[0032]** In addition, since the RING3 protein is known to be localized in the nucleus, the predicted nuclear transport signal of TSB was identified using the PSORT program. As a result, four copies of the nuclear transport signal (in the 488, 489, 575 and 919 positions), each copy consisting of four residues, and two copies of Robins and Dingwall consensus sequence (Robins and Dingwall, (1991) Cell 64: 615-23) (in the 445 and 603 positions) were discovered. Thus, like RING3, nuclear localization of TSB was also indicated.

Example 3 Mapping of TSB

**[0033]** To identify the locus of TSB, DNA from 24 human/rodent single chromosomal somatic cell lines obtained from Coriell Cell Repositories, New Jersey (Dubois and Naylor, (1993) Genomics 16: 315-319) were amplified using primers U (SEQ ID NO: 3) and L (SEQ ID NO: 4).

**[0034]** A panel of single chromosomal hybrid cell lines was screened for the TSB-localized region, using primers U (SEQ ID NO: 3) and L (SEQ ID NO: 4). As a result, the predicted product of 175 bp was amplified only in the cell line GM 13139, a single chromosomal cell line for human chromosome 1. The same primers were used for PCR for a GeneBridge4 radiation hybrid panel (Walter et al., (1994) Nature Genetics 7: 22-28). The binary codes generated by assessing each hybrid as positive or negative for the amplification were compared with the analogous codes for the markers constituting the framework map, using the server located at http://www-genome.wi.mit.edu./cgobin/contig/rhmapper.pl. This procedure was repeated to give independent scores. The two experiments yielded identical binary codes, and TSB was shown to be located on chromosome 1p between markers WI-7719 and WI-3099 (D1S2154) (Fig. 3).

Example 4 Analysis of TSB expression

**[0035]** Northern analysis of 16 normal tissues was conducted using the probe prepared by PCR amplifica-

tion of the testis cDNAs using primers U (SEQ ID NO:3) and L (SEQ ID NO:4). The probe strongly hybridized with mRNA of 3.5 kb and weakly hybridized with that of 4.0 kb (Fig. 4A). This result was consistent with the source testis-specific library of the EST used to identify TSB (Diatchenko et al., (1996) Proc. Natl. Acad. Sci. USA 93: 6025-6030). In addition, the probe cross-hybridized with the two species of mRNA (about 2.0 and 4.5 kb) commonly expressed in these tissues. Since this probe contained the sequence encoding a bromodomain, the transcripts of the two species potentially represent other bromodomain genes. Furthermore, a panel of mRNA derived from eight tumor cell lines was screened along with the panel of the normal tissues, since, several other testis-specific genes, in particular the MAGE family (van der Bruggen et al., (1991) Science 254: 1643-1647) are expressed in tumor tissues. However, expression of TSB was not detected in any of the cell lines tested (Fig. 4B). Likewise, a panel of 10 samples of lung cancer was negative for the TSB expression (data are not shown). The conditions for hybridization were the same as those described in Example 1.

Industrial Applicability

**[0036]** The present invention provides a novel transcriptional regulator having a bromodomain, DNA encoding said transcriptional regulator, a vector carrying said DNA, a transformant expressibly retaining said DNA, an antibody binding to said transcriptional regulator, and a method of screening a compound that binds to said transcriptional regulator. The transcriptional regulator of the present invention is considered to form a family together with a transcriptional regulator RING3 that is thought to be associated with cancer. It is abundantly expressed in the testis. Accordingly, the transcriptional regulator of the present invention and DNA encoding said transcriptional regulator can be used to screen for therapeutics to treat diseases such as cell-proliferative diseases and cancer, particularly testis cancer; diseases attributed to aplasia and dysfunction of sperm; or for contraceptives. Antibodies and other compounds that bind to the transcriptional regulator of the present invention can also be used as therapeutics.

**Claims**

1. A transcriptional regulator having a bromodomain, which comprises the amino acid sequence shown in SEQ ID NO:1, or said sequence wherein one or more amino acids are substituted, deleted, or added.

2. A transcriptional regulator having a bromodomain, which is encoded by DNA hybridizing with DNA comprising the nucleotide sequence shown in SEQ ID NO: 2.

3. A DNA coding for the transcriptional regulator according to claim 1 or 2.

4. A vector comprising the DNA according to claim 3.

5. A transformant expressibly retaining the DNA according to claim 3.

6. A method for producing the transcriptional regulator according to claim 1 or 2, which comprises culturing the transformant according to claim 5.

7. An antibody binding to the transcriptional regulator according to claim 1 or 2.

8. A method of screening a compound having binding activity to the transcriptional regulator according to claim 1 or 2, wherein the method comprises

   (a) contacting a sample with the transcriptional regulator according to claim 1 or 2 and
   (b) selecting a compound having binding activity to the transcriptional regulator according to claim 1 or 2.

9. A compound having binding activity to the transcriptional regulator according to claim 1 or 2, which can be isolated according to the method of claim 8.

10. The compound according to claim 9, which is naturally occurring.

11. A DNA specifically hybridizing with DNA comprising the nucleotide sequence shown in SEQ ID NO: 2 and having at least 15 nucleotides.

Fig. 1

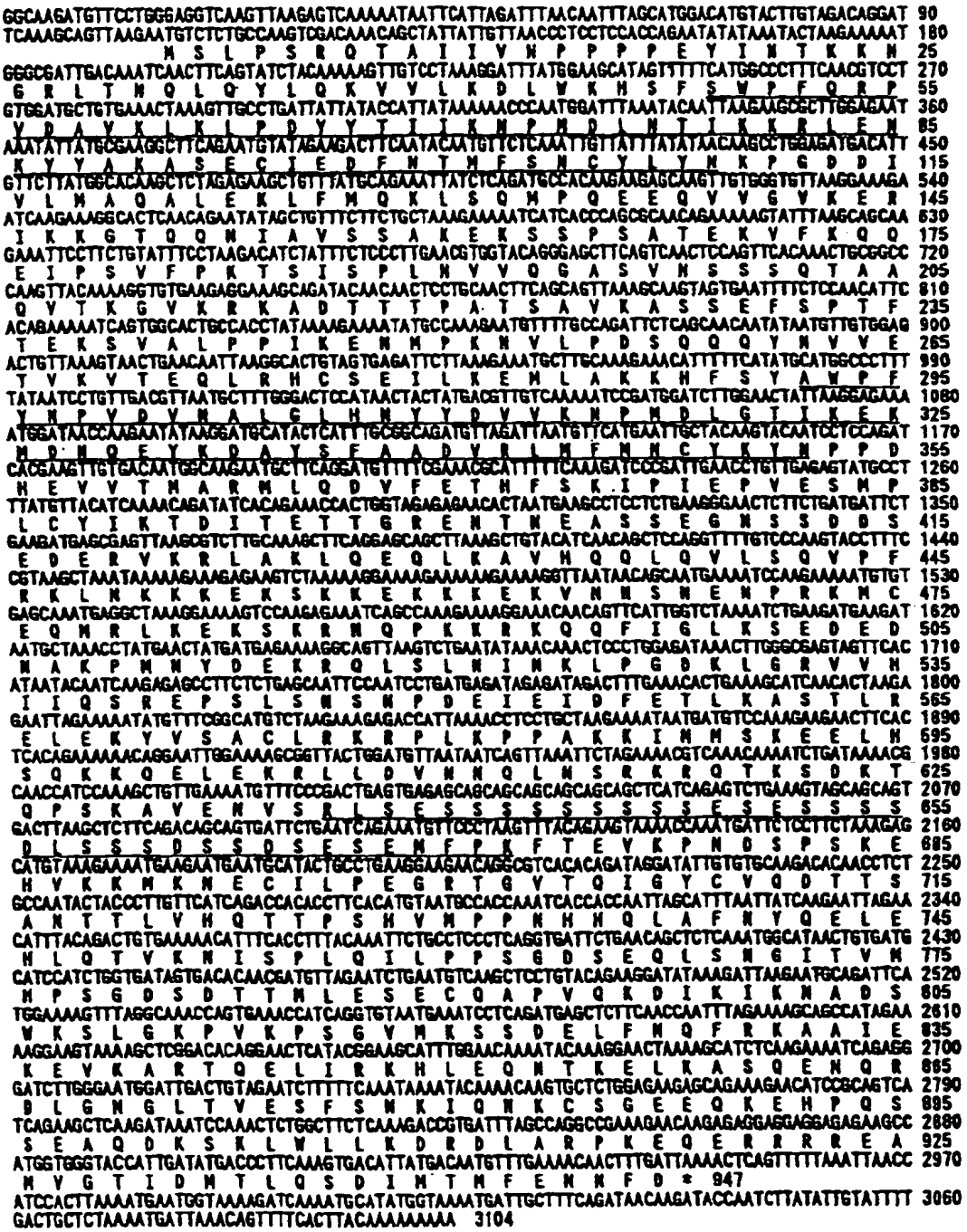


Fig. 2

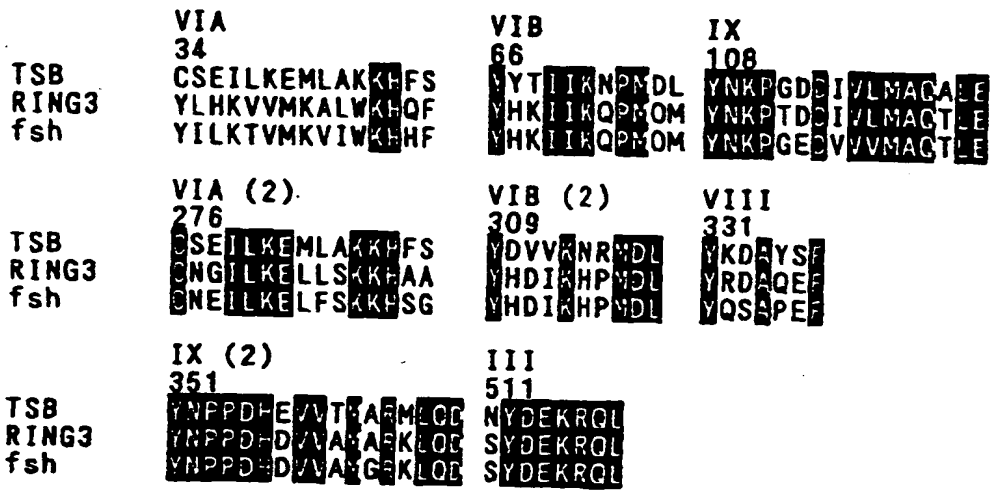


Fig. 3

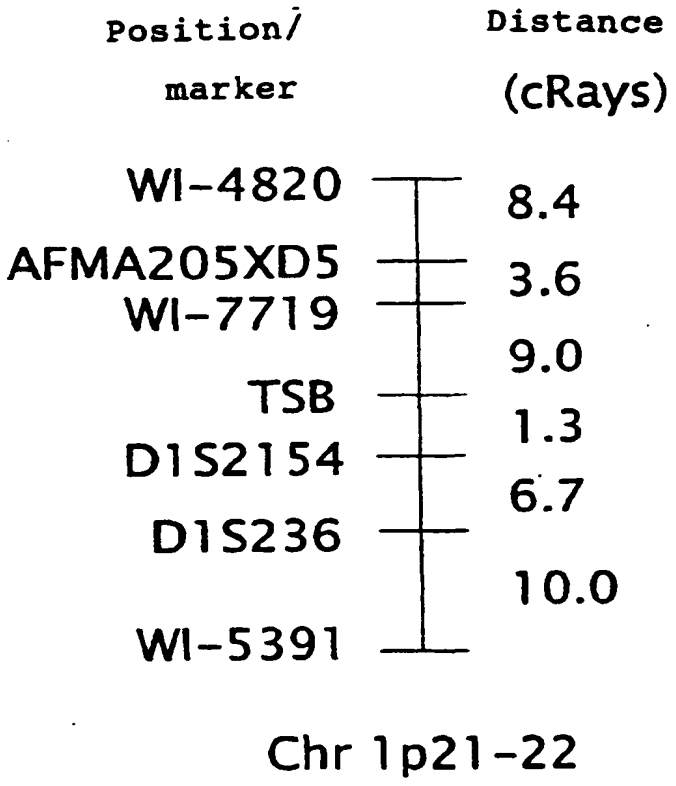
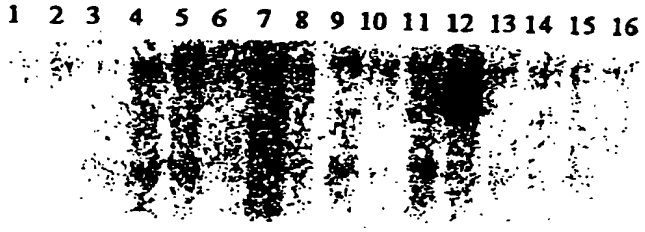


Fig. 4

A




B

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP98/01782

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[6] C12N15/12, C12N15/63, C12P21/02, C07K14/52, C07K14/82, C07K16/24, C07K16/32, C12Q1/02
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[6] C12N15/12, C12N15/63, C12P21/02, C07K14/52, C07K14/82, C07K16/24, C07K16/32, C12Q1/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPI (DIALOG), BIOSIS (DIALOG), GenBank/EMBL/DDBJ/Geneseq

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| PX | Michael, H.J. et al., "Identification and Characterization of BRDT: A Testis-Specific Gene Related to the Bromodomain Genes RING3 and *Drosophila fsh*" Genomics 45 (1997, Nov.) p.529-534 | 1-11 |
| X | Stephan, B. et al., "A homologue of the *Drosophila* female sterile homoetic (fsh) gene in the class II region of the human MHC" DNA Sequence 2 (1992) p.203-210 | 1-11 |
| X | Susan R.H. et al., "The *Drosophila fsh* Locus, a Maternal Effect Homeotic Gene, Encodes Apparent Membrane Proteins" Developmental Biology 134 (1989) p.246-257 | 1-11 |
| A | Nobuo, N. et al., "Prediction of the Coding Sequences of Unidentified Human Genes. II. The Coding Sequences of 40 New Genes (KIAA0041-KIAA0080) Deduced by Analysis of cDNA Clones from Human Cell Line KG-1" DNA Res. 1 (1994) p.223-229 | 1-11 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| July 7, 1998 (07. 07. 98) | July 21, 1998 (21. 07. 98) |
| Name and mailing address of the ISA/<br>Japanese Patent Office<br><br>Facsimile No. | Authorized officer<br><br>Telephone No. |

Form PCT/ISA/210 (second sheet) (July 1992)